

annealing temperature Project N _____

pMCG / dif. enzy / dif. primer / dif

Bo k No. _____

11/28/94

age N _____

purpose: To check at different annealing temp to get rid of mis priming during pMCG amplification

Temp checked 58°, 60°, 62°, 65°

56° gave more non specific bands than 58°. pg 102 -

checked all three primer set { AAT- II new dV
 " " - dV
 " old dV 2728 + 29

Amplified with Tag, Tag + dV, D.V alone.

200 µM dNTP
 1 µM primer
 200 µg template
 2 mM Buffer (from buffer) - used Deep Vent buffer

prepared cocktail for 80 Rx

added primers separately, adding respective enzymes.

when *	1 - 8	Tag	old dV	each combination in duplicate, annealed at different temp 58, 60, 62, 65°.
	9 - 16	}	New dV	
	17 - 24		" - dV	
	25 - 32	T: dV 1:0.01	old dV	94° 3'
	33 - 40	}	new dV	
	41 - 48		" - dV	
	49 - 56	Deep Vent	old dV	30 (94° 30" X 30" 72° 3')
	57 - 64	↓	new dV	
	65 - 72		" - dV	
		1:5 U		72° 10'
				4° 20 min.

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ssed & Understood by me,

Date

12/19/94

Invented by

Recorded by

A. Sivaraman

Date

11/28/94



Project No. _____

110

Book No. _____

TITLE _____

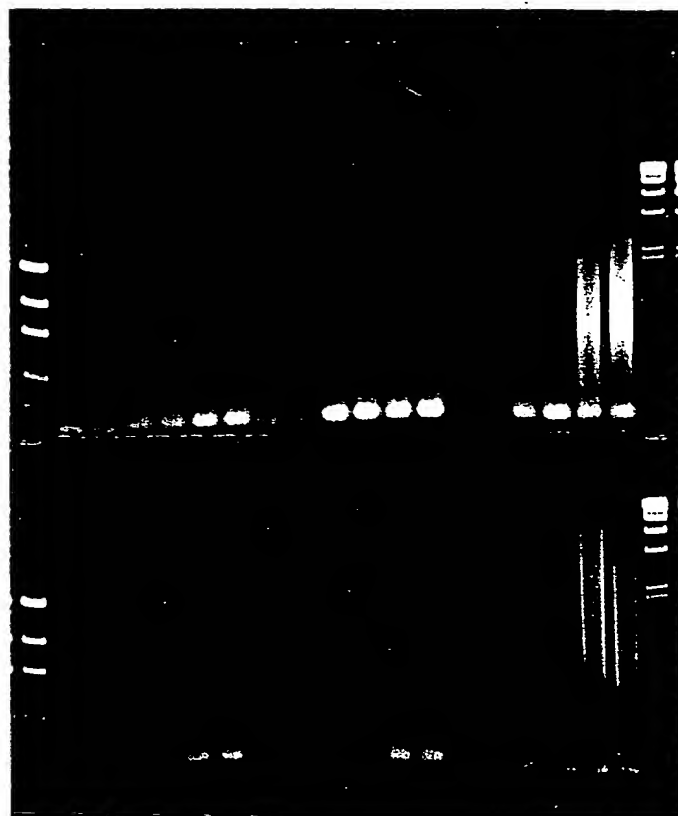
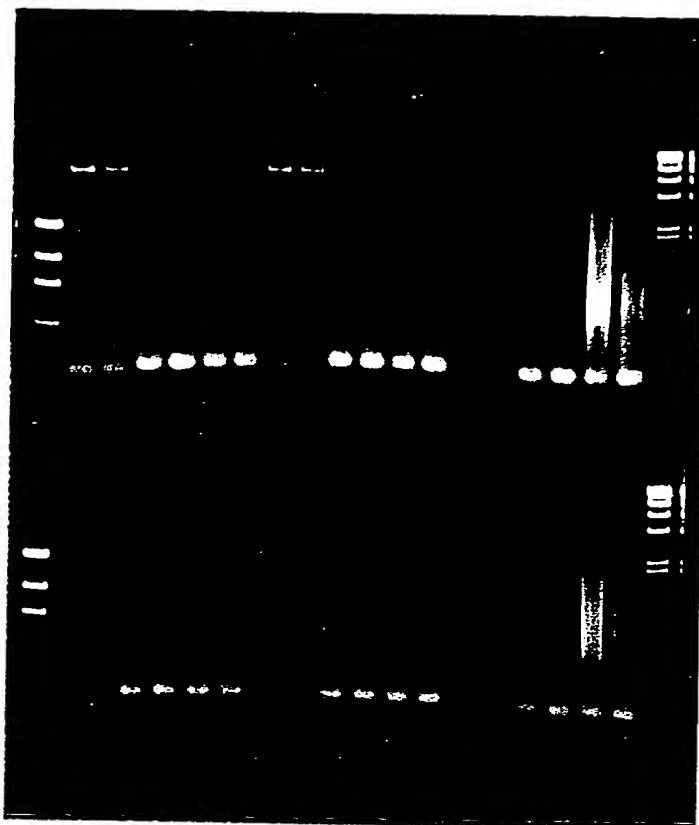
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70.7
0.50 N. 40.50

Tag, D.V.

D.V.

58°

60° *Full*Result: The only thing that worked,

Tag

Tag + D.V.

}

old DV primers at 58°

Deep Vent / non DV / at all anoxic temp remains?

all samples discarded

T Pag N.

With ss & Understood by me,

Date

12/10/64

Invent d by

Record d by

ck. J. K. M. H. M. M.

Date

11/29/64